Given that environmental variables are in constant flux, there are certain to be times when our memories for past experiences are no longer in synch with our current environment and sometimes our reactions based on those memories can be deeply problematic. Irrational fears and post-traumatic stress disorder would seem to be representative of those situations where old memories evoke responses ill-suited to current circumstances. At a very human level, Diaz *et al.* [1] challenge us to understand the specific neurobiological mechanisms underlying reconsolidation and memory updating so we can design therapeutic interventions in which we 'evoke and erase' memories that underlie maladaptive emotional responses.

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Development: CLAVATA1 Joins the Club of Root Stem Cell Regulators

Stem cell maintenance and daughter cell differentiation is essential for root and shoot development. Genetic and physical interaction of the receptor-like kinases ACR4 and CLV1 bring a new player to the field of distal stem cell control in the primary root tip.

Elisabeth L. Williams[1] and Ive De Smet[1,2,*]

Maintenance of stem cells and regulation of daughter cell differentiation are crucial to sustain post-embryonic root and shoot development [1]. In the main root tip, molecular networks have been uncovered regulating stem cell maintenance and daughter cell differentiation. Key regulators of distal stem cell control include auxin, RETINOBLASTOMA-RELATED (RBR), PLETHORAs (PLTs), and WUSCHEL-RELATED HOMEOBOX5 (WOX5) [1,2]. However, in addition to these transcriptional regulators, forward and reverse genetics have pinpointed several receptor-like kinases (RLKs) and small signalling peptides that play an essential role in the root apical meristem [3,4].

RLKs perceive extracellular signals and initiate downstream signalling cascades. The *Arabidopsis* genome encodes more than 600 RLKs, and several of these play an important role in developmental processes [4]. RLKs bind ligands at the extracellular domain, with several binding to small signalling peptides [3,4]. However, in general, few signalling complexes and associated protein–protein interactions have been elucidated [4,5]. Despite the limited knowledge on ligand–receptor interactions in plants, many of these small signalling peptides have been implicated in developmental processes [3]. One of the best characterised *Arabidopsis* signalling peptides is CLAVATA 3 (CLV3), which binds to the RLK CLV1 and controls stem cell fate in the shoot apical meristem [6].

In the *Arabidopsis* root meristem, the membrane-localised RLK ARABIDOPSIS CRINKLY4 (ACR4) exerts control over distal stem cell proliferation and differentiation [7]. ACR4-mediated distal stem cell control has been linked to the small signalling peptide CLAVATA3/EMBRYO SURROUNDING REGION40 (CLE40) [8]. CLE40 regulates expression of *WOX5* resulting in disruption of the distal stem cells. While genetic data suggest that CLE40 acts through ACR4 [8], at present, no biochemical evidence supports this interaction. In this context, the majority of well-characterised (CLE-binding) RLKs contain leucine rich repeat (LRR) extracellular domains [3], but the amino-terminal part of ACR4 comprises unique extracellular 'crinkly' domains that form a β-propeller structure consisting of seven repeats [9]. This different receptor structure suggests that CLE40 might not bind to ACR4 and that ACR4 might interact with a ligand from another family. Due to the interest in uncovering the signal transduction pathway, ACR4 has been well characterised using genetic and cell biological approaches [7,9–12], but physical interaction with closely related ACR4 family member proteins and (auto)phosphorylation have only been analysed *in vitro* [13,14].

A study reported recently in *Current Biology* [15] demonstrates the first direct *in planta* interaction of ACR4 and another protein, CLV1, adding a new player to the distal stem cell maintenance regulatory network in the root (Figure 1). This novel interaction might explain how CLE40 can signal through ACR4, namely as part of an ACR4−CLV1 complex, as it has been demonstrated that CLE40 can bind to CLV1 *in vitro* [16].

The expression of *CLV1* in the root overlaps with expression of *ACR4*,

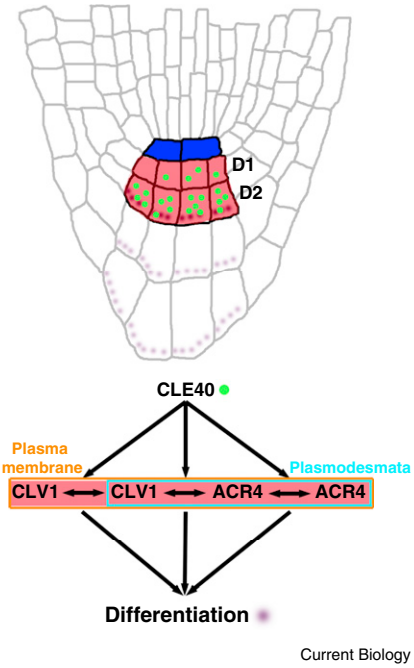

Figure 1. Distal (columella) stem cell regulation by ACR4, CLV1 and CLE40.

Focus on the quiescent centre (blue) and on D1 and D2 layers where ACR4 and CLV1 overlap (red). Formation of homo- and heteromeric complexes occurs at the plasma membrane (orange box) and at plasmodesmata (light blue box). At plasmodesmata, ACR4 forms predominantly homomeric complexes. A gradient of CLE40 (green), possibly directly, acts on ACR4 and CLV1 to control differentiation (purple, starch granules).

suggesting that CLV1 and ACR4 signal in the same cells (Figure 1). Similar to *acr4* and *cle40* mutants, *clv1* displays extra layers of columella stem cells. In addition, *CLV1* expression is dependent on ACR4 and, to some extent, on CLE40. Intriguingly, while CLV1 and ACR4 act together, genetic evidence supports that in the absence of CLV1, an additional (buffering) pathway could be active. This raises the question of how many RLKs are acting in the distal stem cell niche, and how they are connected.

The direct *in planta* interaction between ACR4 and CLV1 was demonstrated through both a split luciferase assay and a more detailed FRET/FLIM analysis. Earlier studies on *in vitro* ACR4 homodimerisation suggest that this occurs via its transmembrane domain (TMD) [13]. Substituting the TMD of ACR4 for the TMD of BAK1 resulted in no interaction with CLV1, supporting specificity and confirming that the interaction of ACR4 with CLV1 *in planta* also occurs via the TMD. To subsequently analyse homo- and heterodimeric complexes of ACR4 and CLV1, Stahl *et al.* [15] used multiparameter fluorescence image spectroscopy (MFIS) as a novel two-dimensional assay based on fluorescence resonance energy transfer (FRET). The results indicated that heterodimers of CLV1 and ACR4 are preferentially formed at the plasma membrane rather than at the plasmodesmata, where homodimerisation of ACR4 was more prevalent (Figure 1). This is in agreement with earlier analyses of CR4, the ACR4 maize orthologue, which showed preferential localisation to the plasmodesmata between aleurone cells [17].

Plasmodesmata, which are plasma membrane lined pores that traverse the cell walls of neighbouring cells and connect their cytoplasms, are important for cell–cell communication [18]. However, very little is known about the control mechanisms at the plasmodesmata. While the distal stem cells are connected to the quiescent centre by plasmodesmata [19], it remains to be uncovered what the specific function of ACR4 (and other RLKs) at the plasmodesmata is. It could be that a signalling hub exists at these pores, and that ACR4 (together with other RLKs) controls movement of molecules through plasmodesmata. But, alternatively, the plasmodesmata could act as 'parking positions' for (inactive) RLKs. Further exploring these options will require identification of (ACR4-dependent) mobile factors (if any) between the columella stem cells and their daughter cells.

Until now, cell identity changes have largely been explained through hormone gradients and transcriptional networks, but it is becoming clear that major signalling events during plant development occur through (reversible) post-translational modifications. In this respect, (de) phosphorylation mediated by kinases and phosphatases has been poorly explored. ACR4, CLV1, and CLE40 provide a unique starting point to integrate the known transcriptional control mechanisms with post-translational modifications regulating stem cell maintenance.

The results obtained by Stahl *et al.* [15] indicate that similarities, with respect to the key players involved in transcriptional and signalling networks, can be drawn between the maintenance of the stem cell niche in the shoot apical meristem and root apical meristem. Members of the same protein families control these processes: the CLV3–CLV1–WUS module influences stem cell maintenance in the shoot apical meristem, whereas in the root apical meristem, the CLE40–ACR4–CLV1–WOX5 module is essential. Since ACR4 is also expressed in the L1 layer of the shoot apical meristem [11], it remains to be investigated if ACR4 also acts in SAM stem cell maintenance. Other RLKs may also play important roles in the signalling networks that specify the stem cell niche in roots. For example, RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2) is another RLK shown to exert control over root and shoot meristem maintenance [20]. However, the shoot apical meristem pathway is not fully recapitulated as CLV2 does not appear to play a role in CLE40-mediated distal stem cell regulation [8]. It is clear that in the future more RLK club members controlling (distal) stem cells in the root can be expected.

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Evolutionary Genetics: Big Effect of a Small RNA

A new study demonstrates that tissue-specific changes in the expression of a microRNA contribute to morphological variation in nature. This and other examples suggest that the evolution of microRNA-regulated gene networks may follow the same general principles as the more familiar regulatory networks controlled by transcription factors.

Artyom Kopp

What is the genetic basis of phenotypic evolution? This question, so obvious yet surprisingly difficult to address, has motivated an ever deeper integration of evolutionary theory with molecular and developmental genetics. A key lesson that emerged from this synthesis is that morphological traits evolve largely through changes in the spatial and temporal regulation of functionally conserved genes [1]. Most of the work to date has focused on the role of evolutionary changes in *cis*-regulatory elements (enhancers) that control tissue-specific transcription. However, gene regulation does not begin and end with transcription; a variety of mechanisms continue to fine-tune protein abundance and activity post-transcriptionally. In this issue of *Current Biology*, Arif *et al.* [2] show that changes in the expression of microRNAs, an important class of post-transcriptional regulators, can also contribute to morphological evolution and can act with the same spatial and temporal specificity as changes in transcriptional networks.

In *Drosophila*, as in other insects, much of the adult cuticle is covered with microscopic trichomes — hair-like cuticular projections secreted by epithelial cells. The spatial distribution of trichomes varies both within and between species [3–5]. In particular, different *Drosophila* species, as well as different wild-type strains of *D. melanogaster*, show extensive variation in the size of the so-called 'naked valley' — a patch of trichome-free cuticle on the second pair of legs [2,4]. Although the adaptive significance of this trait is unknown, rapid evolution of the naked valley makes it a fruitful model for investigating the genetic basis of phenotypic differences between closely related species.

microRNAs (miRNAs) are short, non-coding RNAs that modulate the expression of protein-coding genes by inhibiting translation or inducing mRNA degradation [6–8]. miRNAs are produced by a specialized processing pathway from stem-loop structures contained within longer primary transcripts, and function by interacting with short recognition sites that are typically located in the 3' untranslated regions (UTRs) of protein-coding genes. The specificity of interactions between miRNAs and their targets depends on base pairing between the target site and the 'seed' sequence in the mature miRNA. In animals, most miRNA–mRNA interactions result in only a slight downregulation of the target gene, but some miRNAs can induce an almost complete silencing of a target [6–8].

Arif *et al.* [2] set out to map and identify the genes responsible for intraspecific variation in the size of the naked valley in *D. melanogaster*. In crosses between strains with small and large naked valleys, a single 25-kb genomic region explained over 90% of the phenotypic difference. This region contained only three protein-coding genes whose molecular functions made them unlikely to be involved in trichome development, and one microRNA gene, *miR-92a*. Earlier experiments have shown that overexpression of *miR-92a* in the *Drosophila* wing causes loss of trichomes [9,10], making this miRNA an obvious candidate for the phenotypic variation. No differences were found in the sequence of the mature miRNA, but the strains with a smaller naked valley had higher *miR-92a* expression in the underlying